Observations on the breeding mechanism of *Biophytum candolleanum* Wt.

BY

R. VASUDEVAN NAIR

Victoria College, Palghat, Kerala

(*With a plate*)

Among angiosperms there are 28 families with heterostylous genera (Beryl S. Vuilleumier 1965). Of these, 5 families include trimorphic heterostyled genera. Plants reported to be trimorphic are: *Oxalis* (Mulcahy 1964; Ornduff 1964); *Averrhoa* (Ornduff, Pers. comm.); *Lythrum* (Darwin 1877; East 1940); *Nasaea* (Darwin 1877; East 1940); *Houstonia* (Darwin 1877); *Eichhornia* (Johnson 1924; Uchida 1953); *Pontedaria* (Hazen 1918; Ornduff 1966); *Reussia* (East 1940); *Anielema* (Vogel 1955) and *Biophytum* (Mayura Devi 1964).

In trimorphic hererostyly, a species will have 3 floral forms regarding length of style and height of anthers. This morphological heteromorphism is accompanied by biological specialisation in breeding. Considerable work has been done on the breeding mechanism of dimorphic heterostylous plants since Hildebrand (1866) and Darwin (1877) but relatively less is known about breeding of tristylous plants. Nora Barlow, grand daughter of Darwin, was the first to study the genetic mechanism of heterostylous plants viz. *Lythrum salicaria* and species of *Oxalis*. Since then Hazen (1918), Johnson (1924), East (1940), Lewis (1943), Uchida (1953), Vogel (1955), Bahadur (1963) and Ornduff (1964) have investigated this phenomenon.

Mayura Devi reported in 1964, that in the tristylous *Biophytum sensitivum* DC. floral trimorphism is associated with an incompatibility system. I studied the breeding mechanism of another species of the same genus viz. *B. candolleanum*, described by J. S. Gamble in his Flora of Madras Presidency, vol. i, 94.

Biophytum candolleanum (*B. sensitivium* var. *candolleana* Edgw. & Hook. f.) is a small unbranched annual with pinnately compound, sensitive leaves crowded at the apex of a narrow stem up to 35 cm in height. Peduncles many, axillary, up to 20 cm long but shorter usually. Flowers pedicellate, hermaphrodite, actinomorphic, hypogynous and trimorphic. The 3 forms of flowers are:

long-styled with mid and short stamens,
mid-styled with long and short stamens and
short-styled with long and mid stamens.

Fruits 5-valved loculicidal capsules protected by persisting sepals. Seeds 20-25 per fruit, very small, brownish red, tubercled and arillate. Growing season of the plant is from July to November.

TABLE 1

APPROXIMATE MEASUREMENTS IN MM OF FLORAL PARTS OF *B. candolleanum*

Floral part	Long-styled	Mid-styled	Short-styled
Pedicel (length)	5-10	5-10	5-10
Corolla (diameter)	15	15	15
Sepal (length)	3-3.5	3-3.5	3-3.5
Petal (width)	5-7	5-7	5-7
Corolla (length)	9-11	9-11	9-11
Long stamen	—	4	4
Mid stamen	2.5	—	2
Short stamen	1	1	—
Pistil (height)	3-3.5	2	1

Pollen grains are similar to those of *B. sensitivum* DC., in shape. There is gradation in size of pollen from short stamen to that of long. They form almost a regular series gradually increasing in size.

MATERIALS AND METHODS

Young plants of *B. candolleanum* collected from wild populations at Dharmadam, Tellicherry, Kerala, in July 1969 were used for the study initially. In 1970, plants collected from Palghat, Kerala, were used to repeat some of the crosses done previously. Plants were transplanted in the laboratory, in small wooden boxes. After flowering they were sorted out into long, mid and short-styled forms. Out of 135 plants, 30 were long-styled, 55 mid-styled and 50 short-styled.

Pollination experiments were conducted to find out incompatibility system, if any, in this species. To prevent visit of insects and contamination of pollen, inflorescences were kept enclosed in bags of polythene. In short-styled flowers, sepals had to be cut short to do artificial pollination. Flowers emasculated in the evening were often found withering by next morning. So when emasculation was necessary, it was done at midnight, before dehiscence of anthers. Pollinated flowers were again kept bagged.

POPULATION STRUCTURE

Eliot (1892) recorded a population composition of 25 long: 54 mid: 21 short or about 1:2:1 ratio in the tristylous *Oxalis suksdorfii*. In 1959, Faberge also reported a similar representation of forms in a population of the same species, which was composed of 246 long: 453 mid: 207 short. Ornduff, in 1964 examined ten sample populations. The mid-styled form made up the largest class in four of these.

As the random collection made for transplantation showed difference in proportion of the 3 forms in *B. candolleanum,* 3 more populations were counted to find out the frequency of the 3 types. Result is shown in table 2.

TABLE 2

POPULATION COMPOSITION OF *B. candolleanum*

Date	Place	Total number	Long		Mid		Short
20-8-'70	Palghat	90	20		37		33
12-9-'70	Dharmadam	176	20		80		76
25-9-'70	Dharmadam	200	31		106		63
	Total	466	71	:	223	:	172

It is evident that populations of *B. candolleanum* show what Finney (1952) called anisoplethy (inequality of different floral forms). In all observed cases the mid-styled form makes up the largest class and long-styled the smallest. Short-styled form comes close to the mid-styled, in number. But there is no definite 1:2:1 ratio.

POLLINATION

To find out whether self pollination was taking place, flowers of the 3 forms were kept bagged and unpollinated, for several days. Though flowers opened regularly, there was no fruit-setting even in one. This shows that insect visit is essential for pollination and setting of fruits. Insects like honey-bees, bugs and flies were found visiting the flowers in field.

Self fertility of the 3 forms of flowers was determined by artificial pollination. Flowers opened inside polythene bags were used for this. In the first instance, pollen from the longer set of stamens was transferred to the stigmas of the same flower. Later the same experiments were repeated with pollen from the short stamens. Results are shown below.

A SELFING WITH POLLEN FROM LONGER STAMENS OF SAME FLOWER

Type of flower	Number of flower pollinated	Number of fruits formed	Percentage of fruit setting	Seed range
Long-styled	129	43		
	33	4		
	17	0		
	45	0		
Total	224	47	20.98	5–15
Mid-styled	50	46		
	25	25		
	35	35		
Total	110	106	96.36	15–20
Short-styled	40	40		
	69	52		
	108	68		
Total	217	160	73.73	12–20
B SELFING WITH POLLEN FROM SHORTER STAMENS OF SAME FLOWER				
Long-styled	60	0	0	0
Mid-styled	70	64	91.04	12–20
Short-styled	45	40		
	20	10		
	65	50	76.92	10–20

The above data show that in *B. candolleanum,* the 3 forms of flowers have self sterility in varying degree. The mid-styled form is relatively unspecialised, being fully self-fertile. Short-styled form is also very highly self compatible. Maximum self incompatibility is found in the long-styled form. The unspecialised mid-styled form does not show specificity to type of pollen received; pollen from both sets of stamens are equally effective. But in long-styled flowers, pollen from shorter stamens is totally ineffective.

In heterostylous plants self-pollination is called 'illegitimate' (Darwin 1865). In *Lythrum salicaria,* several tristylous species of *Oxalis,* and many distylous species, the morphological adaptation for cross-pollination is reinforced by incompatibility system, preventing fertilization after illegitimate pollinations. But in some plants such incompatibility does not accompany heterostyly (Hildebrand 1887; Ray & Chisaki 1957). In *B. candolleanum,* there is an incompatibility system, though not perfect. The long-styled form is highly self-incompatible.

CROSS-POLLINATIONS

Pollination between anther and stigma at the same level in different forms of flowers is called 'legitimate' (Darwin 1865), because it is believed that heterostyly is an adaptation to promote cross breeding in hermaphrodite flowers and that such pollinations are the most productive. Where pollen grains of the different sets of anthers are markedly different in morphology, it is easier to find out which type of pollen falls on a stigma. But in *B. candolleanum* it is difficult because the pollen grains show a gradation in size. So crossing was conducted to determine the productivity of pollen from different sets of anthers. Results of some legitimate and illegitimate crosses are given below.

A LEGITIMATE CROSS

Type of cross	Number of pollination	Number of fruits formed	Seed range
Long-styled flower as seed parent			
Long style × Long stamen of short	31	25	
	20	20	
	35	35	
Total	86	80	12–15
Long style × Long stamen of mid	40	40	
	39	39	
Total	79	79	15–25
Mid-styled flower as seed parent			
Mid-style × Mid stamen of long	40	40	
Mid-style × Mid stamen of short	58	54	15–25
Short-styled flower as seed parent			
Short-style × Short stamen of long	60	44	
Short-style × Short stamen of mid	40	28	15–20

From the above data it is evident that *B. candolleanum* is similar to *B. sensitivum* DC. in legitimate crosses. In natural populations there is apparently little difference in the rate of fruit and seed setting between the 3 types of flowers. The comparatively lower rate of fruiting observed in short-styled form in artificial legitimate crossing may possibly be due to imperfection of pollen transfer. In these flowers styles curve out and bend down so that the stigmas are horizontal or even facing down. In emasculated flowers this curving is even more. In hand pollination it is probable that enough pollen grains do not stick on to the stigmas.

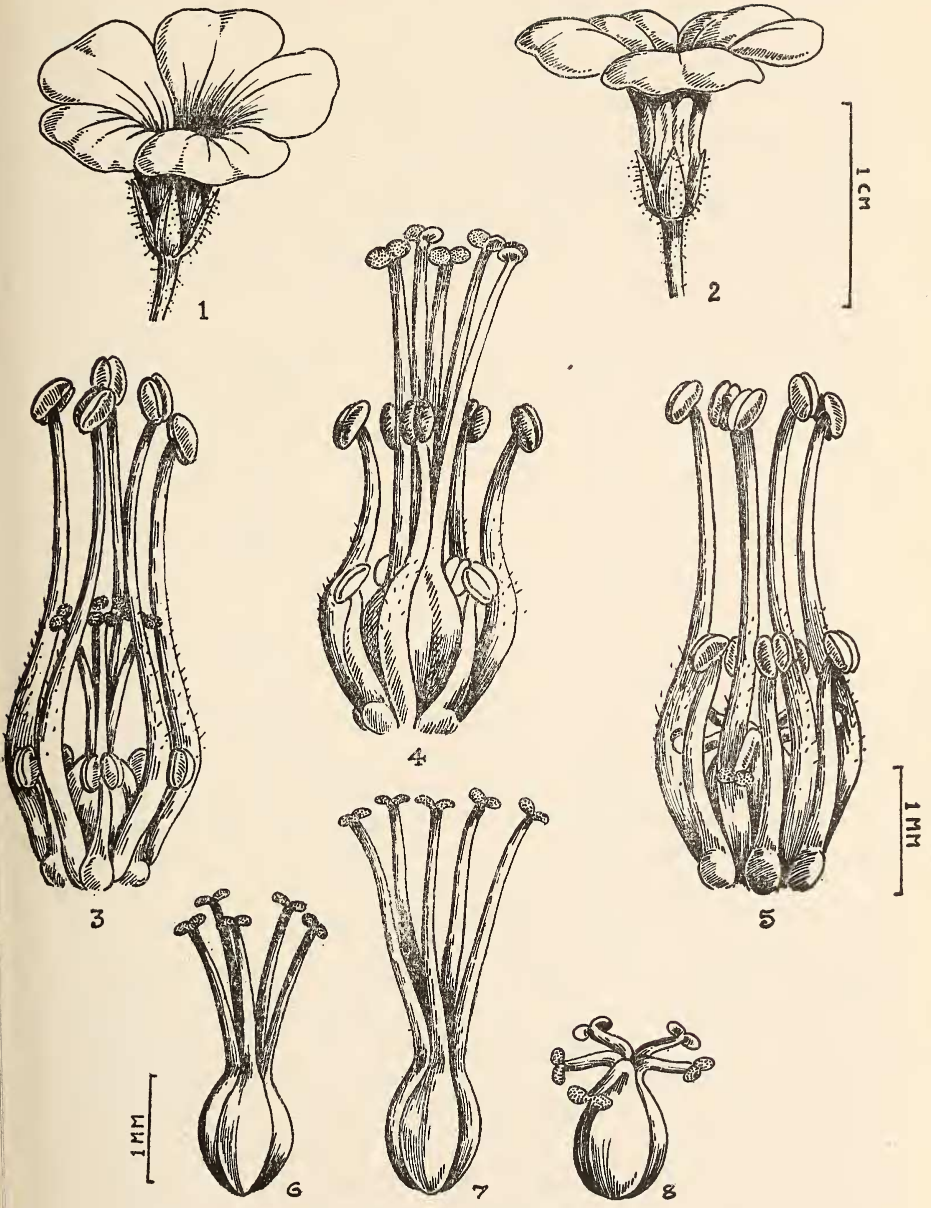
B ILLEGITIMATE CROSSES

Type of cross	Number of pollinations	Number of fruits	Seed range
Long-styled flower as seed parent.			
Long style × Short stamen of mid	60	0	
Long style × Short stamen of short	21	7	
	30	1	
	40	7	
Total	91	15	8–12
Long style × Mid-stamen of long	49	6	
Mid-styled flower as seed parent			
Mid-style × Long stamen of short	29	27	
Mid-style × Long stamen of mid	50	45	
Mid-style × Short stamen of long	28	25	
Mid-style × Short stamen			
Short-style flower as seed parent			
of short	30	29	15–25
Short-styled flower as seed parent			
of long	46	36	15–20
Short style × Mid stamen of mid	46	20	15–20

Percentage of seed formation with respect to pollination was not determined, though the range of variation in number of seeds was noted. It is found that the number of ovules is not constant in all ovaries of even the same type of flower. It varies from 15 to 30. Occasionally, aborted ovules are also seen. Hence, determination of fertility on the basis of number of seeds could be exact only when large number of pollinations are considered. Ready setting of fruits with well formed seeds has therefore been taken as indicating compatibility.

Pollination experiments show that *B. candolleanum* is slightly different from *B. sensitivum* DC. in the case of illegitimate crosses. Short-styled form of *B. sensitivum* DC. is reported to be self compatible only in low degree (Mayura Devi 1964), whereas in *B. candolleanum,* self compatibility is more than 70 per cent. Mid-styled form of this species is even more self compatible than in *B. sensitivum* DC. and unlike in

Nair: *Biophytum candolleanum*



Biophytum candolleanum Wt.

Fig. 1 & 2, Open flowers; Figs. 3, 4, 5, essential organs of mid, long and short-styled flowers; Figs. 6, 7, 8, gynoetium of mid, long and short-styled flowers.

the latter, there is little difference in the productivity of pollen from long and short stamens in selfing. That the mid-styled flower of *Biophytum* is comparatively unspecialised in breeding habits, seems to be supported by the behaviour of the closely allied species *B. reinwardtii* Edgw. & Hook. f.

Specimens of *B. reinwardtii* Edgw. & Hook. f. were collected from Vadakumpad village, Tellicherry, Kerala, in July 1970. The species was identified at Kew, based on herbarium specimens and flowers kept in liquid preservative. This species is very much like *B. sensitivum* DC. in morphology, but smaller, weaker and of shorter duration. It is more susceptible to fungal diseases. Approximate measurements in mm of floral parts are given below.

Length of peduncle	up to 100
Length of pedicel	5-6
Diameter of corolla	7-10
Length of corolla	7-9
Length of sepals	3
Width of petal	4 at apex
Length of stamen:	
Long stamen	2-3
Short stamen	1
Length of pistil	2

In spite of the close similarity to *B. candolleanum* this species is remarkable in its breeding habits. Two populations were observed, collecting more than 100 plants from each. All these plants were of the same kind, being very similar to the midstyled flower of *B. candolleanum* but the styles slightly shorter. Typical long and short-styled forms were not found. The erect styles are held at an angle of about 45° so that their stigmas are just above the short stamens or even touching their anthers. Because of this, at anthesis the stigmas get a good supply of pollen from the short stamens. If this pollen is productive as in *B. candolleanum*, there is no need of insect agent for pollination in this species. To ascertain this, inflorescence were bagged for days on end. Of the 100 flowers which opened inside bags, 96 formed well developed fruits with number of seeds ranging from 20 to 30. Then, 100 bagged flowers were self pollinated with pollen from the long stamens. All of them set fruits, with 20-30 seeds in each. From these observations it is clear that *B. reinwardtii* is a totally self compatible species which is also remarkable in being adapted for self pollination.

B. reinwardtii also shows partial compatibility with *B. candolle-*

anum . Results of some crosses between these two species are as shown below.

Seed parent		Pollen parent	No. of flowers pollinated	No. of fruits	Average No. of seeds
B. candolleanum		*B. reinwardtii*			
Long style	×	Long stamen	125	0	0
Mid style	×	Long stamen	33	17	12.64
Short style	×	Long stamen	21	6	10
B. reinwardtii		*B. candolleanum*			
,,	×	Mid stamen of Long	30	15	16
,,	×	Long stamen of Mid	28	16	12.75
,,	×	Long stamen of Short	24	7	18.4

When long-styled flower of *B. candolleanum* is selected as seed parent, total incompatibility is observed, though cross may be said to be legitimate. But the mid and short-styled flowers show compatibility to some extent. Reciprocal crosses with *B. candolleanum* as pollen parent also show compatibility in varying degree. However, to arrive at definite conclusions further investigation is required.

SUMMARY AND DISCUSSION

Results of artificial breeding in *B. candolleanum* indicate the presence of incompatibility system associated with tristyly and varying in degree. Long-styled form is highly self incompatible. Mid-styled form is totally self compatible. Short-styled form is also very highly self-fertile. Maximum self sterility is seen in the form which has the stigmas far above all the anthers. Forms with at least one set of anthers above the stigmas are highly self fertile.

B. reinwardtii is adapted for self pollination and is totally self fertile. In this species the long and short-styled forms seem to have disappeared, leaving only the mid-styled form with necessary modifications to ensure self pollination.

Mather & de Winton (1941) are of the opinion that incompatibility reaction and morphological dimorphism arose together as one inseparable unit and not as distinct outbreeding mechanisms which are found together because of mutual reinforcement. But this conclusion has since been proved unacceptable. It is generally assumed that a homomorphic multiallelic incompatibility system is primarily in angiosperms (Stebbins 1947, 1950, 1960; Whitehouse 1950; Crowe 1964). Heterostyly which is found in more advanced taxa is believed to be derived from an ancestral multiallelic system either by degeneration of all except two alleles or by the degeneration of all alleles and subsequent